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THE METABOLISM OF TETRALIN IN FISCHER 344 RATS

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

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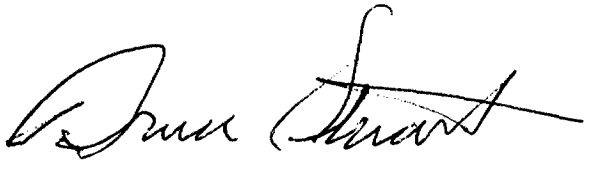

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Certain hydrocarbons have been shown to cause nephrotoxicity in male rats. Because of tetralin's unique structural and electrical character, tetralin metabolism in male and female Fischer 344 rats and its effects on renal damage were evaluated. Male and female Fischer 344 rats were intragastrically dosed every other day with tetralin over a 14 day period. Additional Fischer rats were pretreated intraperitoneally with either corn oil, sodium phenobarbital, 3-methylcholanthrene or SKF 525-A and then dosed with tetralin intragastrically every other day over a 14 day period. When compared with male control rats, male rats exposed to tetralin exhibited increased cytoplasmic hyaline droplets in proximal convoluted tubular epithelial cells which were indicative of toxic injury. Additionally, foci of cellular degeneration were present within proximal convoluted tubules. All pretreated male rats, except for 2 rats pretreated with 3-methylcholanthrene, exhibited renal lesions. Exposed and control female rats did

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not display any renal damage. The tetralin metabolites found in the urine of all male and female exposed rats were: 1-tetralol, 2-tetralol, 2-hydroxy-1-tetralone, 4-hydroxy-1-tetralone, 1,2-tetralindiol, and 1,4-tetralindiol. Tetralin metabolites were not found in the kidney extracts of male and female rats dosed only with tetralin. However, tetralin, 1-tetralol, 2-tetralol, and 1-tetralone were found in the kidney extracts of male rats pretreated with corn oil, sodium phenobarbital, and SKF 525-A. Only one male rat pretreated with 3-methylcholanthrene had any tetralin metabolites detected in its kidney extract and they were 1-tetralol and 1-tetralone. This male rat was also the only 3-methylcholanthrene pretreated rat to exhibit renal lesions.

PREFACE

This research was conducted in the Toxicology Branch, Toxic Hazards Division, Air Force Aerospace Medical Research Laboratory from July 1984 through December 1985. It was performed in support of Project 6302, "Occupational and Environmental Toxic Hazards in Air Force Operations;" Task 630210, "Toxicology of Alternate Aircraft Fuels;" 63021001, "Health Environmental Effects of Alternate Aircraft Fuels." This document was submitted and accepted for the Master of Science Degree requirements of Lt Beth M. Llewellyn.

Acknowledgement of appreciation is made to Drs Wayne Carmichael and Mark Mamrack (Wright State University) for their expert help; to SSgt Gayle McDonald (Toxic Hazards Division) for her significant technical contributions; to Mrs Pamela Reynolds (Toxic Hazards Division) for typing many times this seemingly endless project; and to HM3 Fred Campbell (Naval Medical Research Institute, Toxicology Detachment) for illustrating the schematic diagrams of the unilobular kidney.

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I. INTRODUCTION

In the early 1980s, widespread use of petroleum products and public emphasis on toxic hazard assessment influenced the American Petroleum Institute to sponsor a study to determine what potential adverse health effects, if any, were presented to people who came in contact with gasoline under normal circumstances. The study, conducted by the International Research and Development Corporation, examined the inhalation effects of unleaded gasoline in mice and rats. The unexpected occurrence of dose-related degrees of kidney damage, including renal neoplasia, in male rats evoked concern. Renal lesions were not observed in female rats or in males or females of other test species.

The results of the gasoline study, reported in 1983 by the Universities Associated for Research and Education in Pathology, prompted extensive investigations by the scientific community on unleaded gasoline and other hydrocarbon-based compounds in an attempt to ascertain the mechanism of nephrotoxicity in the male rat. In 1983, a Workshop on the Kidney Effects of Hydrocarbons was conducted in Boston. The material presented during this workshop is published in a book entitled "Renal Effects of Petroleum Hydrocarbons" (Advances in Modern Environmental Toxicology, Vol VII, M. A. Mehlman, ed., Princeton Scientific Publishers, Inc., 1984). The concluding remarks from this workshop urge the continuation of investigations of nephrotoxic hydrocarbons. Additionally, at the 1985 meeting of the Society of Toxicology, a continuing education course in renal toxicology, with special emphasis on hydrocarbon nephrotoxicity was offered.

In 1985, The American Cancer Society estimated that 19,700 Americans, approximately two-thirds of them male, would develop renal cancer and 8,900 would die from the disease. The relevance of nephropathy observed in male rats exposed to various hydrocarbons to the occurrence of renal neoplasia in man is of concern.

Anatomic and physiologic differences, especially between the rat and man, were observed when normal kidneys from animals of different species were compared. However, these comparative studies have not yet explained why, of all the animal species studied, only male rats develop kidney lesions and renal carcinomas. Therefore, the suitability of using the male rat as a model for human risk assessment is in question.

The observation of hydrocarbon-induced nephrotoxicity and carcinogenicity in male rats raises important questions regarding the potential hazard of numerous hydrocarbon-based compounds. Despite the widespread use of petroleum products, the potential health effects of many hydrocarbons remain largely uncharacterized. Therefore, to aid in the elucidation of a mechanism of toxicity, it is imperative that experiments be conducted to evaluate the structure-activity relationships of various hydrocarbons and their involvement in nephrotoxicity.

NORMAL RENAL STRUCTURE AND FUNCTION

A discussion of normal renal structure and function is necessary in order to understand hydrocarbon-induced nephrotoxicity. The kidney

and its components are diagrammed in Figure 1. If a kidney is sectioned sagittally, two regions can be distinguished, an outer cortex and an inner medulla. A transitional area called the corticomedullary junction exists between the cortex and the medulla where elements from both regions are found. The medulla is shaped like an inverted pyramid which has its base juxtaposed to the innermost border of the cortex and its apex (papilla) facing the pelvis. Each medullary pyramid and the cortical tissue that encase it constitute a lobe. Some species, incuding the rat, have unilobular kidneys where the narrow papilla projects into the flared end of the ureter, known as the renal pelvis.

The kidney is a compound gland formed of uriniferous tubules. Each uriniferous tubule is composed of a nephron and a collecting duct system. The nephron produces the urine while the collecting duct system collects, concentrates, and transports this fluid to the pelvis, where it leaves the kidney. The nephron consists of a Bowman's capsule, a proximal convoluted tubule, an ascending and descending loop of Henle, and a distal convoluted tubule.

Histologic evaluation of the renal cortex and the renal medulla displays regular structural patterns. Sections of the renal cortex show bands called medullary rays which consist of descending and ascending limbs of the loop of Henle and straight collecting tubules. Medullary sections reveal a visible separation of the medulla into outer and inner zones. The outer zone contains the loops of Henle of short nephrons and straight collecting tubules while the inner zone contains the loops of Henle of long nephrons and straight collecting ducts and papillary ducts.

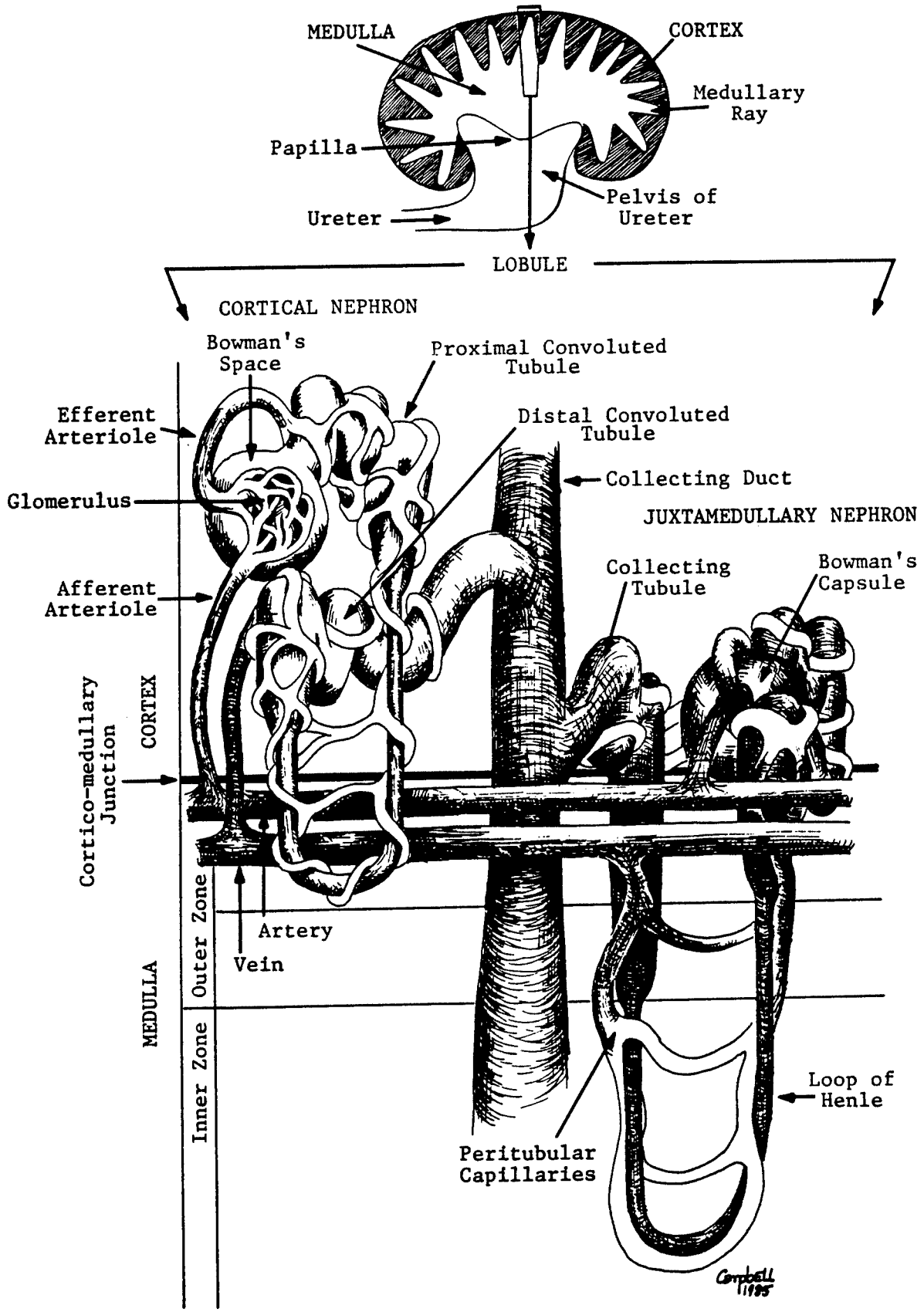


FIGURE 1. SCHEMATIC DIAGRAMS OF A UNILOBULAR KIDNEY.

The nephron is the functional unit of the kidney and contains within Bowman's capsule a special filtering mechanism called the glomerulus. The glomerulus is supplied with an arteriole of the renal artery. The arteriole enters Bowman's capsule as the afferent arteriole and branches into a network of capillaries which form part of the glomerulus. These capillaries rejoin and emerge from Bowman's capsule as the efferent arteriole. The efferent arteriole divides into a system of capillaries (peritubular capillaries) which tightly surrounds all of the tubular parts of the nephron. Blood in the peritubular capillaries drains into venules, which combine to form the renal vein.

As blood flows through the capillaries of the glomerulus, the pressure of the blood causes fluid to filter into Bowman's capsule and then into the proximal tubule. The function of the different segments of the kidney tubule is to reabsorb certain substances, in varying degrees, depending on the needs of the body. In the proximal tubule there is active transport of filtered glucose, amino acids, sodium, and ions from the tubule into the proximal tubular epithelial cells. These solutes are processed inside the epithelial cells, and transported into the peritubular capillaries. The result is that water passes out of the tubule by osmosis while urea and other wastes are concentrated in the tubule. Also, low molecular weight proteins that were filtered at the glomerulus are transported into the proximal tubular epithelium where lysosomes degrade the proteins into their constituent amino acids. These amino acids diffuse through the basal membrane of the cell, and into the peritubular capillaries.

In the loop of Henle a counter-current fluid mechanism and the presence of various hormones increase the concentration of sodium chloride in the tubular fluid. As the tubular fluid flows into the distal tubule the permeability of the distal tubule and collecting duct is increased under hormonal control and reabsorption of water follows by osmosis. The result is that fluid leaving the collecting duct to enter the pelvis of the kidney is concentrated urine.

LESIONS CHARACTERIZING HYDROCARBON-INDUCED NEPHROTOXICITY

Male rats exposed to some hydrocarbons develop dose-related nephropathies which are not observed in female rats and control rats or in the males and females of other animal species. These lesions seem to be dependent on the progressive accumulation of excessive quantities of resorbed, undigested protein in the cytoplasm of proximal tubular cells (Bruner et al., 1983). This undigested protein usually appears as a spherical, homogeneous body termed a hyaline droplet. The inability of the cell to efficiently degrade and export these resorbed protein droplets results in greatly engorged phagolysosomes which precede cell death and exfoliation (Bruner et al., 1983). In contrast, most other nephrotoxins exert their effects by interfering with essential metabolic processes, causing cell damage without excessive accumulations of resorbed proteins (Cheville, 1983). An overview of the pathologic findings from the kidneys of laboratory animals subjected to acute, subchronic, and chronic hydrocarbon exposures is presented.

A. Acute Exposures: Male rats exposed to certain hydrocarbons for up to 14 days develop excessive cytoplasmic hyaline droplets in the cells of the proximal convoluted tubules. When exposures are continued

for more than 14 days, renal tubules near the corticomedullary junction accumulate cellular debris and cortical segments exhibit hyperplastic changes.

B. Subchronic Exposures: Most subchronic exposures have been based on the 90-day continuous inhalation of a specified hydrocarbon fuel. Male rats sacrificed immediately following various subchronic exposures exhibit a distinct increase in cytoplasmic hyaline droplets in the proximal tubular epithelial cells throughout the cortex. Additionally, tubular segments near the corticomedullary junction are focally dilated and filled with coarsely granular, eosinophilic debris. These dilated, plugged tubular segments are thought to represent that region of the proximal tubule where it narrows to enter the descending limb of the loop of Henle (Bruner et al., 1983). Transmission electron microscopy has demonstrated that tubular plugs consist of cell debris and that hyaline droplets are compatible with membrane-bound accumulations of protein in phagolysosomes. Other renal structures, including glomeruli, are morphologically unremarkable with both light and electron microscopy. Pathologic evaluation of animals subchronically exposed and then held for long-term, post-exposure evaluation revealed tubular degeneration consistent with "old-rat nephropathy" (explained below).

C. Chronic Exposures: Many chronic hydrocarbon studies have consisted of one-year intermittent inhalation exposures. Histopathologic examination of the male rat kidneys following these one-year exposures has revealed a significant increase in primary renal tumors. Other kidney changes noted were an increase in lesions typical of "old-rat nephropathy".

One problem inherent in long-term nephrotoxicity studies is that lesions known as "old-rat nephropathy" often obscure pathologic evaluations. "Old-rat nephropathy" is a common degenerative kidney disease predominantly seen in the male rat. By careful examination of tissues, differences between "old-rat nephropathy" and hydrocarbon-induced nephropathy can be distinguished. Foremost among these differences is that degenerative changes are more severe in hydrocarbon exposed male rats. This increased severity is accompanied by the presence of mineralized debris in the medullary tubules and hyperplasia of the surface epithelium over the renal papillus which usually is not present in "spontaneous" "old-rat nephropathy" (Bruner et al., 1983).

PREVIOUS HYDROCARBON NEPHROTOXIC STUDIES

Carpenter et al. (1975, 1977) studied animal responses to several solvents such as Stoddard solvent, a petroleum distillate composed of straight and branched chain hydrocarbons, naphthenes, and benzene derivatives. In each study, single to multiple low doses of solvent produced noticeable renal lesions in the kidneys of male rats compatible with those observed in male rats acutely exposed to hydrocarbons.

General toxicology studies conducted at the Harry G. Armstrong Aerospace Medical Research Laboratory (AAMRL) evaluated petroleum and shale-derived JP-5, a jet fuel composed of aliphatic and aromatic hydrocarbons with the majority of the straight-chain hydrocarbons being between C_{10} and C_{15} (Gaworski, 1979). Purebred beagles, rats, and mice were continuously exposed in inhalation chambers to 150 or 750 mg/m^3 of JP-5 for 90 days. Both males and females of each specie were exposed,

along with equal numbers of controls. Following exposure, all of the dogs and one-third of the rodents from each group were sacrificed. The remaining rodents were held under observation for 19 months. At that time, one-half of the rodents were sacrificed and the other half were held until the mortality of each group reached 90%. Histopathologic evaluations were performed on all animals and renal changes, noticed only in the male rats, were similar to the lesions of male rats subchronically exposed to other hydrocarbon-based compounds.

Since this initial short-term study, the U.S. Air Force with the U.S. Navy and the Toxic Hazards Research Unit at AAMRL, have conducted numerous investigations of distillate propellants (McNaughton et al., 1983). JP-8, JP-4, and diesel fuel marine (DFM) are several petroleum and shale-derived distillates which have been studied in 90-day inhalation experiments. JP-4 is a low boiling point, highly volatile hydrocarbon mixture much like gasoline. JP-8 is a mixture of hydrocarbons of intermediate boiling point and volatility and is similar to the civilian jet fuel, A-1. DFM is a mixture of long chain aliphatic hydrocarbon compounds with a small portion of aromatic hydrocarbons. For those studies in which pathologic evaluations have been completed, the kidney lesions observed were similar to those lesions found in animals subchronically exposed to hydrocarbon-based compounds.

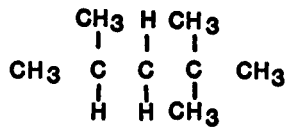
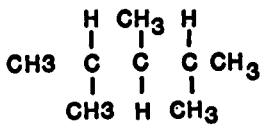
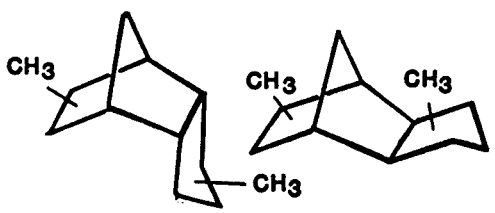
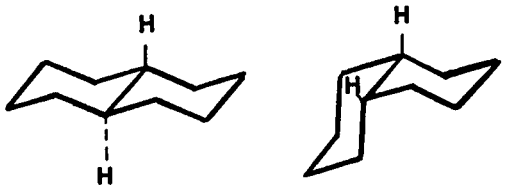
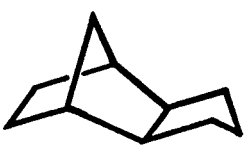
Studies conducted to characterize the nephrotoxic activity of unleaded gasoline in the male rat indicated that the toxic insult occurred predominantly from the fractions containing saturated, branched aliphatic compounds (Halder et al., 1984). Therefore, numerous studies have been initiated to determine the toxicity of

"pure" hydrocarbons. The majority of the exposures performed have been either short-term oral dosings or long-term inhalation exposures.

Short-term oral dosings have been performed using 2,2,4-trimethylpentane, and 2,3,4-trimethylpentane as well as the cyclic hydrocarbons RJ-4 fuel (perhydromethylcyclopentadiene), JP-10 fuel (tricyclodecane), and decalin (decahydronaphthalene). Kidneys from male rats exposed by gavage to these pure hydrocarbons have exhibited the same pathologic changes as seen with the distillate fuels (Bruner, et al., 1983). Table 1 summarizes the results from short-term exposure of male Fischer 344 rats to various hydrocarbons.

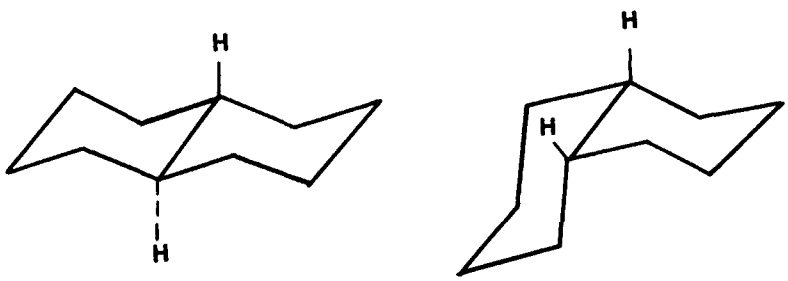
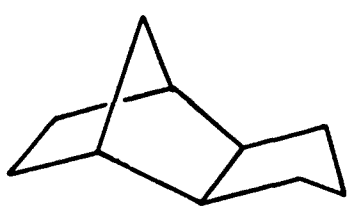
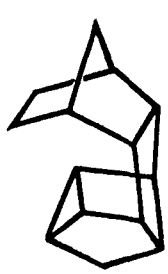
Long-term inhalation exposures have been accomplished primarily with the pure hydrocarbons, decalin, JP-10, and RJ-5 [endo-endo-dihydrodinorbornadiene] (Bruner et al., 1983). Table 2 summarizes the pathology observed after long-term inhalation exposure to pure hydrocarbons. Studies utilizing JP-10 and RJ-5 included one year intermittent inhalation exposures (6 hr/day for 5 days/wk) followed by long-term, post-exposure holding. As in the other hydrocarbon fuel studies, histopathologic evaluation of kidneys from animals exposed to JP-10 and RJ-5 displayed typical chronic hydrocarbon-induced nephrotoxicity in male rats. The most significant histopathologic finding in male rats exposed to JP-10 and held post-exposure was the presence of nine primary renal cell carcinomas and one poorly differentiated malignant renal neoplasm in 50 exposed rats as compared to only one renal cell carcinoma in controls. In animals exposed to RJ-5, a high incidence of renal tumors in male rats was also seen. Of the 65 male rats exposed to 150 mg/m^3, four renal cell adenomas and five renal cell carcinomas were found. Only one renal cell carcinoma

TABLE 1. SHORT-TERM ORAL EXPOSURE OF MALE FISCHER 344 RATS TO SPECIFIC (PURE) HYDROCARBONS

AGENT	DOSE	EXPOSURE LENGTH	RENAL LESIONS
2,2,4-Trimethylpentane 	0.09,0.3, 1.0 mL/kg	8 doses over 24 Days	Hyaline droplets and necrosis of proximal tubular epithelium. Impacted cellular detritus at the corticomedullary junction.
2,3,4-Trimethylpentane 	0.09,0.3, 1.0 mL/kg	8 doses over 24 Days	Hyaline droplets and necrosis of proximal tubular epithelium. Impacted cellular detritus at the corticomedullary junction.
RJ-4 Fuel 	0.09,0.3, 1.0 mL/kg	8 doses over 24 Days	Hyaline droplets and necrosis of proximal tubular epithelium. Impacted cellular detritus at the corticomedullary junction.
Decalin 	2.5 g/kg	7 doses over 14 Days	Hyaline droplets and necrosis of proximal tubular epithelium. Impacted cellular detritus at the corticomedullary junction.
JP-10 	0.09,0.3, 1.0mL/kg	8 doses over 14 Days	Hyaline droplets in proximal tubular epithelium.

Adapted From: Bruner, R. H., and Pitts, L. L.: Nephrotoxicity of hydrocarbon propellants to male Fischer-344 rats. Proc 13th Ann Conf Environ Toxicol, Air Force Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio, 337-349 (1983).

TABLE 2. LONG-TERM INHALATION EXPOSURE OF MALE FISCHER 344 RATS TO SPECIFIC (PURE) HYDROCARBONS

AGENT	DOSE	EXPOSURE LENGTH	RENAL LESIONS
Decalin ($C_{10}H_{18}$) 	5,50 ppm	90 days continuous	Subchronic: Cytoplasmic hyaline droplets and necrosis of proximal tubular epithelium. Impacted tubules at corticomedullary junction. Oncogenic: Accentuated tubular degeneration, medullary mineralization and urothelial papillary hyperplasia.
JP-10 ($C_{10}H_{16}$) 	100 ppm	1 year industrial (6h/d, 5d/wk)	Ten primary renal cell carcinomas in 50 rats.
RJ-5 ($C_{14}H_{20}$) 	30,150 mg/m3	1 year industrial	Low Dose: One renal cell carcinoma. High Dose: Four renal cell adenomas and five renal cell carcinomas in 65 rats.

Adapted From: Bruner, R. H., and Pitts, L. L.: Nephrotoxicity of hydrocarbon propellants to male Fischer-344 rats. Proc 13th Ann Conf Environ Toxicol, Air Force Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio, 337-349 (1983).

was found in 65 male rats exposed to 30 mg/m^3 of RJ-5. No renal cell tumors were identified in control animals. These data indicate that both JP-10 and RJ-5 cause renal cell tumor formation and nephrotoxicity in male rats. A 90-day inhalation experiment was conducted with decalin, and the hydrocarbon-induced nephrotoxicity observed was virtually identical to that of the 90-day distillate inhalation experiments.

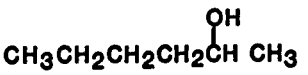
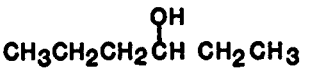
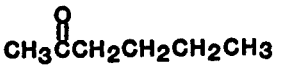
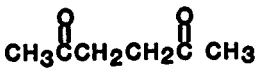
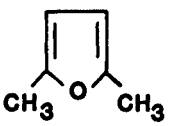
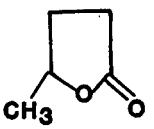
Alden et al. (1983) used both short-term oral gavage treatments and intermittent inhalation exposures to characterize the effect of decalin on male rats. Using two-dimensional electrophoresis and immunofluorescent techniques, Alden et al. demonstrated hyaline droplet accumulation to consist of alpha 2u globulin, a protein previously identified by Roy and Neuhaus (1966). Alpha 2u globulin is a sex-dependent protein synthesized in the liver under testosterone induction and is found in the urine of young adult male rats (Irwin et al., 1971). Levels of alpha 2u globulin are extremely low in the female rat and undetected in humans at this time (Kloss, 1985). Alpha 2u globulin is a low molecular weight protein (18,000 to 20,000 daltons) and, after its synthesis in the liver, is filtered by the kidney glomeruli. A large portion of the filtered protein is then reabsorbed by the proximal convoluted tubules and catabolized by lysosomes into constituent amino acids. The increased appearance of alpha 2u globulin in the urine of male rats is thought to result from the inability of the proximal tubules to reabsorb the protein (Kloss, 1985). Alden et al. (1983) postulated that accumulation of hyaline droplets in epithelial cells is due to an alteration in the handling of alpha 2u globulin.

HYDROCARBON METABOLISM STUDIES

The structure of a xenobiotic determines the type of biotransformation it undergoes and also determines the intermediates or final products formed. The metabolite formed may react with cellular components instead of being excreted. Therefore, an understanding of the metabolic handling of various hydrocarbons is necessary in order to describe more fully the nature of hydrocarbon-induced nephrotoxicity. It is proposed that the interaction of hydrocarbon metabolites with indigenous proteins such as alpha 2u globulin might compromise tubular cell protein catabolism, resulting in the kidney lesions observed (Kloss et al., 1985). As a result, research has focused on the isolation and identification of hydrocarbon metabolites.

Exposures to n-hexane and n-heptane, major constituents of industrial solvent mixtures, have been shown to cause polyneuropathy in man (Casarett and Doull, 1980). Several general hydrocarbon toxicity studies have been performed with n-hexane and n-heptane to characterize their metabolism and role in neurotoxicity (Perbellini et al., 1982; Bahima et al., 1984). These studies have indicated that peripheral neuropathies can be attributed to the metabolite 2,5-hexanedione. However, no nephrotoxicity was reported in any of these straight-chain hydrocarbon toxicity studies. Other nephrotoxicity studies using acyclic aliphatic compounds have indicated that only saturated, branched compounds induce renal lesions in male rats (Kloss, 1985). The urinary metabolites of n-hexane and n-heptane are listed in Table 3. It is important to note that the primary metabolites of n-hexane and n-heptane are alcohols and hydroxyketones.

TABLE 3. n-HEXANE AND n-HEPTANE METABOLITES IDENTIFIED IN THE URINE OF RATS

n-HEXANE METABOLITE	STRUCTURE
2-Hexanol	
3-Hexanol	
Methyl n-butyl ketone	
2,5-Hexanedione	
2,5-Dimethylfuran	
γ -Valerolactone	

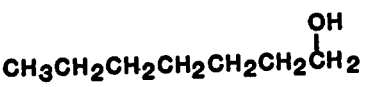
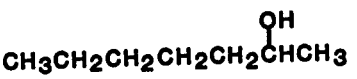
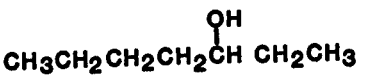
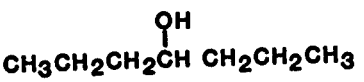
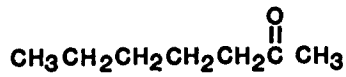
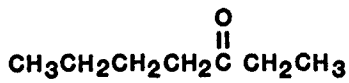
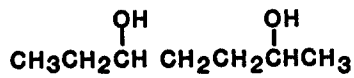
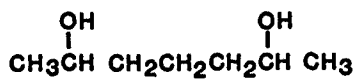
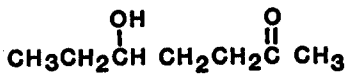
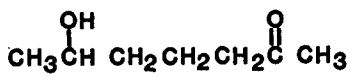
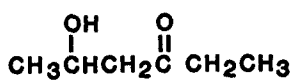
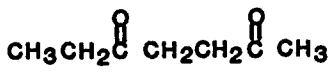
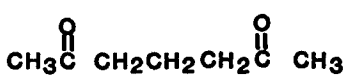
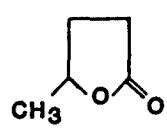
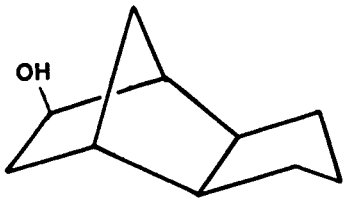
n-HEPTANE METABOLITE	STRUCTURE
1-Heptanol	
2-Heptanol	
3-Heptanol	
4-Heptanol	

TABLE 3 (CONTINUED)

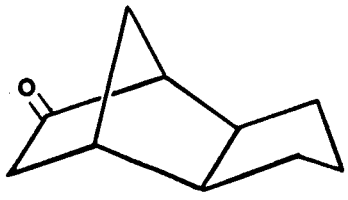
n-HEPTANE METABOLITE	STRUCTURE
2-Heptanone	
3-Heptanone	
2,5-Heptanediol	
2,6-Heptanediol	
5-Hydroxy-2-heptanone	
6-Hydroxy-2-heptanone	
6-Hydroxy-3-heptanone	
2,5-Heptanedione	
2,6-Heptanedione	
γ-Valerolactone	

Olson et al. (1985) orally dosed Fischer 344 rats with the branched, aliphatic hydrocarbon, 2,2,4-trimethylpentane, every other day for 14 days. Trimethylpentane (TMP) is a major component of gasoline and the standard reference fuel for indicating "octane rating". Kidney tissues from TMP exposed animals manifested the same pathologic changes as observed in tissues from animals acutely exposed to distillate fuels. Analysis of urinary metabolites indicated that 2,2,4-trimethylpentane is excreted primarily as a carboxylic acid derivative and to a lesser degree as a monosubstituted alcohol. The urinary metabolites of TMP are shown in Table 4. There were no hydroxyketones or diols detected in the urine of TMP exposed rats.

Although numerous studies have evaluated the metabolism of straight and branched chain hydrocarbons, only a few studies have been conducted to evaluate the metabolism of cyclic hydrocarbons. Inman et al. (1983) studied the metabolism of the C_{10} hydrocarbon fuel JP-10 in Fischer 344 male rats. The metabolites are shown in Figure 2. The only urinary metabolite isolated was 5-hydroxy-JP-10. Since JP-10 produced renal lesions in male rats, one kidney from each of the rats was removed and homogenized for metabolite analysis. The homogenized kidney extract yielded only one derivative of JP-10, 5-keto-JP-10. Renal damage was not detected in female Fischer 344 rats dosed with JP-10 and 5-keto-JP-10 was not isolated from their kidney extracts.



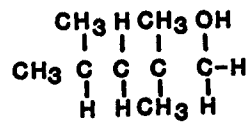
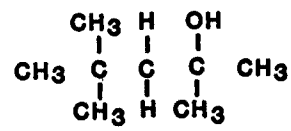
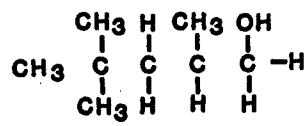
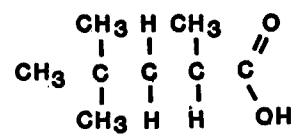
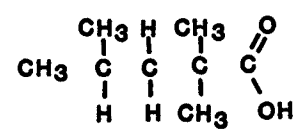
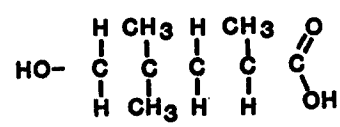
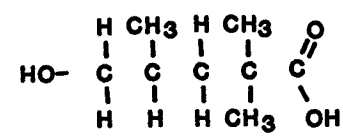
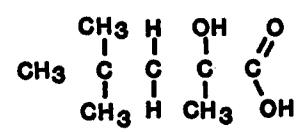
5-Hydroxy-JP-10



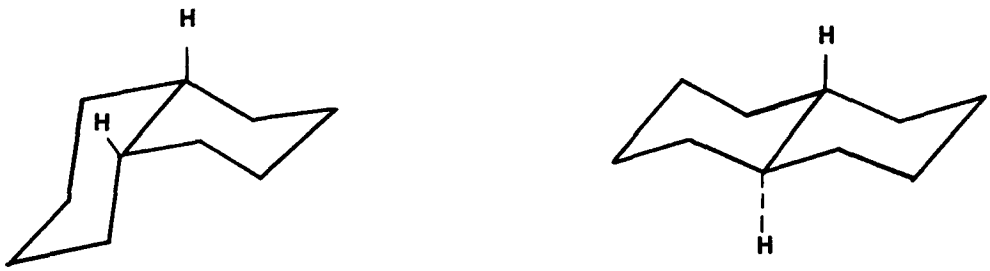
5-Keto-JP-10

FIGURE 2. JP-10 METABOLITES

TABLE 4. 2,2,4-TRIMETHYLPENTANE METABOLITES IDENTIFIED IN THE URINE OF MALE RATS

2,2,4-TRIMETHYLPENTANE METABOLITE	STRUCTURE
2,2,4-Trimethyl-1-pentanol	
2,4,4-Trimethyl-2-pentanol	
2,4,4-Trimethyl-1-pentanol	
2,4,4-Trimethyl-1-pentanoic acid	
2,2,4-Trimethyl-1-pentanoic acid	
2,4,4-Trimethyl-5-hydroxy-1-pentanoic acid	
2,2,4-Trimethyl-5-hydroxy-1-pentanoic acid	
2,4,4-Trimethyl-2-hydroxy-1-pentanoic acid	

Olson et al. (1985) studied the metabolism of cis- and trans-decalin in Fischer 344 rats. The stereoisomers of decalin are shown in Figure 3. Decalin is a component of motor fuels and lubricants and is a solvent for fats, resins, oils and waxes. Like JP-10, decalin is a cyclic hydrocarbon containing 10 carbons. Cis- and trans-decalin produced typical hydrocarbon nephrotoxicity in male rats, while female and control rats exhibited no renal pathology.



Cis-Decalin Trans-Decalin

FIGURE 3. DECALIN STEREOISOMERS

Olson et al. identified and isolated the urinary metabolites of cis- and trans-decalin from male and female Fischer 344 rats. The relative amount of each metabolite detected is listed in Table 5. In both male and female Fischer 344 rats, the principal urinary metabolite of cis-decalin was cis,cis-2-decalol and the major urinary metabolite of trans-decalin was trans,cis-2-decalol. The principal metabolic difference between male and female rats treated with cis-decalin was the presence of cis,cis-1-decalol in the urine of male rats but not in the urine of female rats. It was also noted that the metabolite cis,trans-1-decalol, although found in the urine of both male and female rats, was present in relative larger quantities in the male rat. In the case of trans-decalin, the metabolite trans,trans-1-decalol was found in the urine of male rats and not in the female rat urine. Also, the metabolite trans,cis-2-decalol was found in larger quantities in male rat urine.

TABLE 5. URINARY METABOLITES DETECTED IN MALE AND FEMALE FISCHER 344 RATS TREATED WITH CIS- AND TRANS- DECALIN

	RELATIVE AMOUNT OF METABOLITE DETECTED (GC PEAK AREA)	
	MALE RAT	FEMALE RAT
CIS-DECALIN		
Cis,Cis-2-Decalol	4.0	3.3
Cis,Cis-1-Decalol	1.0	ND[a]
Cis,Trans-1-Decalol	2.6	1.0
TRANS-DECALIN		
Trans,Cis-2-Decalol	5.7	1.0
Trans,Trans-1-Decalol	1.0	ND[a]

[a] = Non-detectable

Analysis of kidney extracts from male and female Fischer 344 rats dosed with cis- and trans-decalin also proved interesting. Renal damage was observed in all of the male rats dosed with cis-decalin, and the presence of cis-2-decalone was detected in homogenized kidney extracts (Olson et al., 1985). For the male rats dosed with trans-decalin, 5 of the 6 rats showed kidney lesions and trans-2-decalone was present in their kidney extracts. The single male rat which presented no renal damage following trans-decalin dosing had no detectable trans-2-decalone in its kidney extract. None of the female Fischer 344 rats dosed with cis- or trans-decalin had kidney damage and 2-decalone was absent from their kidney extracts. The presence of ketones in the kidney extracts of male rats exposed to cyclic hydrocarbons suggests that the ketone could be the causative agent of renal damage or a chemical marker indicating the occurrence of renal damage.

TETRALIN

Since the cyclic hydrocarbons JP-10 and cis-and trans-decalin both produced renal lesions in male Fischer 344 rats, an evaluation of other cyclic hydrocarbons was considered necessary. These evaluations were designed to determine if all cyclic hydrocarbons, regardless of structure, are capable of eliciting nephrotoxic effects, or if by altering the hydrocarbons structure, the nephrotoxic effect can be reduced or eliminated. A logical cyclic hydrocarbon to study for rat nephrotoxic effects was tetralin (tetrahydronaphthalene) (See figure 4). Tetralin, like decalin, contains ten carbons and is composed of two fused six-membered rings. However, the structural and electronic character of tetralin is unlike decalin.

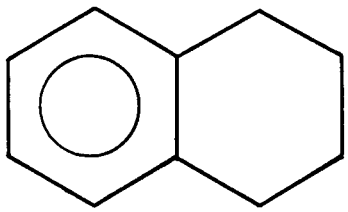


FIGURE 4. TETRALIN

Structurally, the aromatic ring of tetralin causes that part of the molecule to be planar while the aliphatic portion of the molecule remains non-planar. Both of the decalin isomers are composed of two, fused cyclohexane rings which exist in a non-planar chair configuration.

Electronically, the aromatic ring of tetralin will activate the alpha-carbons towards oxidation. The structural and/or electrical differences may preclude or facilitate the metabolism of tetralin to potentially toxic molecules.

A study of urinary metabolites from male and female Fischer 344 rats exposed to tetralin may provide information about positions on the tetralin molecule which undergo oxidation and the effect they have on renal toxicity. Additionally, the presence or absence of tetralin metabolites in kidney extracts, along with histopathology of kidney tissues, should help clarify the function of the metabolites in eliciting renal damage.

PROBLEM STATEMENT

The objectives of this research project were to orally dose male and female Fischer 344 rats every other day with tetralin over a 14 day period and collect and evaluate the following information:

1. The effects of tetralin dosing on animal weight gain as compared to sham controls dosed with water;
2. Histopathologic evaluation of kidney and liver tissues from exposed animals in order to determine damage and note any differences which appear to be a function of sex;
3. Identification of urinary metabolites of tetralin in dosed Fischer 344 rats and differences in structure and relative amounts between males and females;

4. Identification of tetralin metabolites isolated from the kidneys of tetralin-exposed Fischer 344 rats and any differences between males and females;
5. The effects of cytochrome oxidase system inhibitors and inducers on tetralin metabolism, resultant metabolite production, and the appearance of renal lesions with respect to male and female Fischer 344 rats.

II. METHODS

MATERIALS

Twenty-four Fischer 344 male rats approximately 4 months in age and weighing 311 $\pm$ 18 g and twenty-four female rats approximately 4 months in age and weighing 185 $\pm$ 6 g were purchased from Charles River Breeding Laboratories, and randomly allocated to exposure groups (see Table 5). From these forty-eight animals, six male rats and six female rats were given 0.5 mL/kg (485 mg/kg) body weight neat tetralin intragastrically on alternate days over a 14 day period. Equal numbers of control rats for both sexes were given 0.5 mL/kg body weight of water intragastrically. The remaining 12 male and 12 female rats were allocated to four exposure groups with three male rats and three female rats in each group. Rats from each of the four exposure groups were pretreated intraperitoneally with either enzyme modifiers or a control substance (vehicle) for a prescribed number of days, and then dosed with 0.5 mL/kg body weight of neat tetralin intragastrically on alternate days over a 14 day period. The pretreatment chemicals were sodium phenobarbital dissolved in saline, 3-methylcholanthrene suspended in corn oil, and SKF 525-A dissolved in saline. The control substance used was corn oil. Sodium phenobarbital and 3-methylcholanthrene pretreatment groups were given the chemical intraperitoneally at 102 mg/kg body weight and 27 mg/kg body weight, respectively, daily for three days prior to tetralin exposure. Rats pretreated with SKF 525-A were given 25 mg/kg body

weight of the chemical intraperitoneally one day prior to exposure. "Positive" control rats were given 2 mL/kg body weight of corn oil daily for three days before exposure. Following exposure to tetralin, the rats were placed in metabolism cages for 24 and 48 hour urine collection, after which they were housed in plastic cages. All rats were weighed daily. Water and feed (Ralston Purina Co., St Louis, MO) were available ad libitum. At the end of the 14 day dosing period, rats were placed in metabolism cages for overnight urine collection and then sacrificed by anesthetic overdose. One kidney and the median lobe of the liver from each rat were harvested for histopathologic evaluation. Tissues were immersed in 10% neutral buffered formalin, imbedded in paraffin blocks, and cut into sections 6 microns in thickness. They were then mounted on glass slides and stained with routine hematoxylin and eosin. The other kidney was used for metabolite analysis. Urine and kidney samples were frozen until metabolite analyses were performed.

ANALYTICAL PROCEDURES

Isolation of tetralin metabolites from urine and kidney samples was accomplished using the extraction technique of Yu (1985). Urine collected from study animals was allowed to thaw at room temperature. Equal volume aliquots from the same urine sample were adjusted to a pH of 4.0. A 0.5 mL volume of glucuronidase/sulfatase (Calbiochem, La Jolla, CA) was added to one aliquot of each sample which was then heated to 37°C with shaking for 16 hours. Following incubation, aliquots were cooled to room temperature, and then filtered separately through Clin-Elut tubes (Analytichem International, Harbor City, CA) using methylene

chloride as eluent. Second aliquots, which were not enzyme treated, were filtered in a similar manner. The kidneys saved and frozen for metabolite analysis were allowed to thaw, homogenized in distilled water, and processed using the same procedure as for the urine. The eluates from both the urine and kidney samples were individually concentrated by evaporation under nitrogen in preparation for gas chromatographic analysis.

Gas chromatography was used to analyze the metabolites and extracted components from the urine and kidney samples. A Hewlett-Packard 5880A gas chromatograph equipped with a flame ionization detector was used. A 10 m x 0.2 mm I.D. carbowax 20M fused silica capillary column (Hewlett-Packard, Palo Alto, CA) provided good separation for urinary extracts and a 15 m x 0.25 mm I.D. carbowax 20M fused silica capillary column (Supelco, Bellefonte, PA) was used for analysis of kidney extracts. For analysis of urine extracts, oven temperature was programmed from 60° to 170°C at 5°/min after an initial delay of one minute and a hold at final temperature for 30 minutes. A temperature program from 100° to 190°C was used for kidney extracts with a final holding time of 35 minutes. For both urine and kidney analyses, detector and injection port temperatures were 250° and 200°C, respectively. Helium was used as the carrier gas with a split ratio of 20:1. The linear velocity was 23.5 cm/sec at 100°C for the 10 meter column and 34.2 cm/sec at 100°C for the 15 meter column.

Metabolite identification was accomplished using a Hewlett-Packard gas chromatograph/mass spectrometer (GC/MS). The mass spectrometer used was a quadrupole instrument and ionization was obtained by electron impact at a voltage of 70 eV with an ion source temperature of 200°C.

Helium was used as the carrier gas with an injection port temperature of 200°C. For urine extract analysis, the same 10 m x 0.2 mm I.D. carbowax 20M fused silica capillary column was used with a linear velocity of 21 cm/sec at 100°C. Oven temperature was held at 60°C for one minute and then programmed at 5°/min to 170°C with a final holding time of 30 minutes. A 20 m x 0.25 mm I.D. carbowax 20M fused silica capillary column (Supelco, Bellefonte, PA) was used for kidney extract analysis with a linear velocity of 32.5 cm/sec at 100°C. Column temperature was programmed from 100° to 190°C at 5°/min with initial hold of one minute and final time of 30 minutes. Comparison of mass spectra fragmentation patterns with the fragmentation patterns from purchased or synthesized compounds confirmed identification of urinary and kidney metabolites.

CHEMICALS USED FOR METABOLITE IDENTIFICATION

In order to identify tetralin metabolites in urine and kidney extracts, chemicals were purchased from the Aldrich Chemical Co., Milwaukee, Wisconsin. Chemicals directly used for analysis were: tetralin, 1-tetralol, 1-tetralone, 2-tetralone, 5,6,7,8-tetrahydro-1-naphthol, 5,6,7,8-tetrahydro-2-naphthol. The following chemicals were used in the synthesis of possible tetralin metabolites: tetraethylammonium hydroxide, t-butylhydroperoxide, osmium tetroxide, 1,2-dihydronaphthalene, m-chloroperoxybenzoic acid, trimethylsilyl chloride, triethylamine, chromyl chloride, 1,4-naphthoquinone, and lithium aluminum hydride.

SYNTHETIC PREPARATIONS

2-Tetralol

A mixture of lithium aluminum hydride (1.9g, 0.05 mole) in 200 mL of ether was added to a solution of 1-tetralone (15g, 0.1 mole) in 50 mL

of ether. The solution was refluxed for 4 hours. After cooling, 50 mL of water was added dropwise with stirring. The ether solution was decanted off and dried over anhydrous sodium sulfate. Removal of the ether left a solid which when recrystallized from petroleum ether yielded 2-tetralol (10.8 g, 0.074 mole, 74% yield), mp 50-1°C (Lit mp 50-1°C) (Pickard, 1912).

1,2,3,4-Tetrahydronaphthalene-cis-1,2-diol

A 250 Erlenmeyer flask was charged with 100 mL of t-butyl alcohol, 7.5 mL of 10% aqueous tetraethylammonium hydroxide and 1,2-dihydronaphthalene (5g, 0.022 mole). After cooling to 0°C in an ice bath, 9 mL of 90% t-butylhydroperoxide and 5 mL of 0.5% osmium tetroxide in t-butyl alcohol were added. The resulting solution was stirred for 2 hours at 0°C and placed in a refrigerator overnight. After 50 mL of 5% sodium bisulfite was added, the solution was allowed to warm to room temperature with stirring. Removal of the t-butyl alcohol under reduced pressure yielded a residue which was extracted with ether. The ether solution was passed through a 10 cm column of neutral alumina. Evaporation of the ether gave 1,2,3,4-tetrahydronaphthalene-cis-1,2-diol (2.1 g, 0.013 mole, 58% yield), mp 100-2°C (Lit mp 102°C) (Strauss, 1921).

1,2,3,4-Tetrahydronaphthalene-trans-1,2-diol

A solution of 85% m-chloroperoxybenzoic acid (24 g, 0.115 mole) and 200 mL of methylene chloride was cooled to 0°C in a 500 mL Erlenmeyer flask. 1,2-Dihydronaphthalene (10 g, 0.08 mole) in 25 mL of methylene chloride was added dropwise. The solution was allowed to warm to room temperature and then was stirred for 12 hours. The solution was cooled

to 0°C and the precipitated m-chlorobenzoic acid was filtered off. The filtrate was washed consecutively with 25 mL of water, 2-50 mL portions of 10% sodium hydroxide, and 25 mL of water. After drying over sodium sulfate, the methylene chloride was removed under reduced pressure. The 3,4-dihydronaphthalene-1,2-oxide (6.3 g, 0.043 mole, 53% yield) was distilled at 120-2°C (15 Torr), (Lit bp 124-5°C) (13 Torr) (Strauss, 1921). A mixture of 3,4-dihydronaphthalene-1,2-oxide (4 g, 0.027 mole) in 10 mL of ether was added dropwise to a solution of lithium aluminum hydride (1 g, 0.037 mole) and 100 mL of ether. The solution was refluxed for 4 hours. Upon cooling, the solution was hydrolyzed. Separation and drying of the ether layer yielded 1,2,3,4-tetrahydronaphthalene-trans-1,2-diol (3.8 g, 0.023 mole, 86% yield), mp 110-2°C (Lit mp 112°C) (Strauss, 1921).

2-Hydroxy-1-tetralone

1-tetralone (36 g, 0.25 mole) was added to a solution of trimethylsilyl chloride (32.6 g, 0.30 mole) and triethylamine (60.6 g, 0.60 mole) in 100 mL of dimethylformamide. The solution was refluxed for 48 hours. Upon cooling, the solution was diluted with 200 mL of pentane. After washing with 2-300 mL portions of cold 5% sodium bicarbonate solution, the solution was dried over sodium sulfate. Distillation yielded 1-trimethylsilyloxy-3,4-dihydronaphthalene (47 g, 0.22 mole, 76% yield), bp 90-3°C (30 Torr), (Lit bp 78-9°C) (17 Torr) (House, 1969). Chromyl chloride (2 g, 0.012 mole) in 10 mL of methylene chloride was added to a stirred solution of 1-trimethylsilyloxy-3,4-dihydronaphthalene (2.2 g, 0.01 mole) and 20 mL of dry methylene chloride under nitrogen at -78°C. After stirring for 30 minutes at -78°C, the solution was added to a 25 mL cold 5% sodium

bisulfite solution and stirred for 15 minutes. The resulting green solution was neutralized with a 5% sodium bicarbonate solution. After filtration through a Buchner funnel, the solution was extracted with 2-100 mL portions of methylene chloride. The solution was then chromatographed on an alumina column to give 2-hydroxy-1-tetralone (1.3 g, 0.008 mole, 67% yield), mp 58-60°C (lit mp 58-60°C) (Vedejs, 1978).

4-Hydroxy-1-tetralone

1,4-naphthoquinone (3.0 g, 0.019 mole) was reduced with lithium aluminum hydride (1.0 g, 0.027 mole) in 250 mL of ether using a Soxhlet extractor. The hydride solution was then hydrolyzed. The ether layer was separated and immediately subjected to chromatography on an alumina column. Evaporation of the ether yielded a viscous oil which showed hydroxyl and ketone peaks in the infrared spectrum. Mass spectrometry showed a molecular ion m/z = 162 (Boyland, 1951). By-products of the reaction were 1,2,3,4-tetrahydronaphthalene-cis- and trans-1,4-diols.

To confirm the presence of hydroxy-ketones, the urine from three male rats exposed to tetralin was pooled and added to 2 mL of methylene chloride. This solution was then reduced using a mixture of lithium aluminum hydride (1.0 g, 0.026 mole) in 300 ml of ether. Stirring was continued overnight at room temperature. The ether layer was separated and dried over anhydrous sodium sulfate. After evaporation of the ether, the residue was taken up in methylene chloride and analyzed by GC/MS.

III. RESULTS AND DISCUSSION

EFFECT OF TETRALIN ON PHYSIOLOGIC PARAMETERS

The effect of tetralin dosing on the body weights of male and female Fischer 344 rats is summarized in Table 6 and Table 7. Weight loss occurred in all tetralin-exposed animals and was continuous throughout the exposure period. On the average, control rats dosed with water (negative control rats) either maintained their original body weight or gained weight slightly during the exposure period. Statistical comparisons of weight changes that occurred in dose groups of the same sex were performed using the Student's t-test. Each dose group was compared separately to all other dose groups and was considered statistically different at $p \leq .05$. Because of experimental design constraints, the number of rats used to study the effects of enzyme modifiers was limited and, therefore, sample sizes were not optimum for statistical analysis. Nevertheless, statistical analyses were used to emphasize the extreme weight changes observed between pretreated rats and non-pretreated rats.

Male positive control rats (rats pretreated with corn oil prior to tetralin exposure) exhibited a $7.4 \pm 0.2\%$ average weight loss which was statistically significant when compared to the $0.46 \pm 1.8\%$ average weight gain of negative control rats. There was also a statistically significant difference between the weight loss of positive control rats and the $5.4 \pm 1.6\%$ weight loss of rats exposed only to tetralin. There

TABLE 6. THE EFFECT OF TETRALIN DOSING EVERY OTHER DAY FOR 14 DAYS ON THE BODY WEIGHT OF MALE FISCHER 344 RATS

GROUP & TREATMENT	EXPOSURE ROUTE AND DOSE	INITIAL WEIGHT (g)[a]	TERMINAL WEIGHT (g)[a]	WEIGHT CHANGE (g)[a] (%BW±SD)
1. Negative Control Water	0.5 mL/kg i.g.	294 ± 16 (6)	295 ± 15 (6)	+ 1.3 ± 5.3 (+.46 ± 1.8)[f,h]
2. Tetralin	0.5 mL/kg i.g.	300 ± 11 (6)	284 ± 10 (6)	-16 ± 7.9 (-5.4 ± 2.6)[g,h]
Enzyme Modifiers				
3. Positive Control Corn Oil[b] Tetralin	2.0 mL/kg i.p. 0.5 mL/kg i.g.	324 ± 10 (3)	300 ± 7 (3)	-24 ± 1.0 (-7.4 ± 0.2)[f,g,h]
4. Sodium Phenobarbital[c] Tetralin	102 mg/kg i.p. 0.5 mL/kg i.p.	325 ± 10 (3)	295 ± 17 (3)	-29 ± 17 (-9.0 ± 5.4)[h]
5. SKF 525-A[d] Tetralin	25 mg/kg i.p. 0.5 mL/kg i.g.	323 ± 10 (3)	300 ± 8 (3)	-23 ± 2.5 (-4.9 ± 4.0)[h]
6. 3-MC[e] Tetralin	27 mg/kg i.p. 0.5 mL/kg i.g.	328 ± 15 (3)	238 ± 8 (3)	-90 ± 22 (-27 ± 5.6)[h]

a = Mean ± S.D. (N)
b = Given daily for three days prior to tetralin exposure.
c = Given daily for three days prior to tetralin exposure
d = Given one day prior to tetralin exposure
e = 3-Methylcholanthrene given daily for three days prior to tetralin exposure.
f, g, h = Each dose group was compared statistically to all other dose groups. Values with identical superscripts were significantly different from each other at $p \leq .05$ using the Student's t-test.

TABLE 7. THE EFFECT OF TETRALIN DOSING EVERY OTHER DAY FOR 14 DAYS ON THE BODY WEIGHT OF FEMALE FISCHER 344 RATS

GROUP & TREATMENT	EXPOSURE ROUTE AND DOSE	INITIAL WEIGHT (g)[a]	TERMINAL WEIGHT (g)[a]	WEIGHT CHANGE (g)[a] (%BW±SD)
1. Negative Control Water	0.5 mL/kg i.g.	183 ± 7 (6)	185 ± 4 (6)	+2.2 ± 5.2 (+1.2 ± 2.9)[g]
2. Tetralin	0.5 mL/kg i.g.	186 ± 7 (6)	176 ± 6 (6)	-9.3 ± 2.1 (-5.0 ± 1.1)
Enzyme Modifiers				
3. Positive Control Corn Oil[c] Tetralin	2.0 mL/kg i.p. 0.5 mL/kg i.g.	181 ± 7 (3)	173 ± 8 (3)	-8.3 ± 2.9 (-4.6 ± 1.7)[g]
4. Sodium Phenobarbital[d] Tetralin	102 mg/kg i.p. 0.5 mL/kg i.p.	186 ± 1 (3)	178 ± 1 (3)	-8.7 ± 0.9 (-4.6 ± 0.5)
5. SKF 525-A[e] Tetralin	25 mg/kg i.p. 0.5mL/kg i.g.	186 ± 3 (3)	179 ± 3 (3)	-10 ± 4.1 (-4.1 ± 0.8)
6. 3-MC[f] Tetralin	27 mg/kg i.p. 0.5 mL/kg i.g.	189 ± 11 (3)	157 ± 3 (2)[b]	-27 ± 4.5 (-15 ± 1.7)

a = Mean ± S.D. (N)
b = One female rat died.
c = Given daily for three days prior to tetralin exposure.
d = Given daily for three days prior to tetralin exposure
e = Given one day prior to tetralin exposure
f = 3-Methylcholanthrene given daily for three days prior to tetralin exposure.
g = Each dose group was compared statistically to all other dose groups. Values with identical superscripts were significantly different from each other at $p \leq .05$ using the Student's t-test.

was no statistically significant difference between the weight changes of tetralin exposed rats and negative control rats. Finally, rats pretreated with 3-methylcholanthrene before tetralin dosing exhibited a weight loss of 27 $\pm$ 5.6% which was statistically significant when compared to all other dose groups.

The weight loss observed in rats pretreated with corn oil is possibly due to the irritating properties of the chemical on the gastrointestinal system. The substantial weight loss of rats pretreated with 3-methylcholanthrene resulted in a poor physical condition at time of necropsy. Polycyclic aromatic hydrocarbons have been reported to cause gastroenteric distress upon ingestion (Clayton, 1981). Since 3-methylcholanthrene is a polycyclic aromatic hydrocarbon, it is possible that the severe weight loss observed in rats pretreated with this chemical was due to gastroenteric distress.

Female positive control rats lost an average 4.6 $\pm$ 1.7% of their original body weight which was statistically different from the 1.2 $\pm$ 2.9% average weight gain of negative control rats. As with the male rats pretreated with corn oil, the weight loss in positive control rats could have been caused by the irritating properties of the chemical on the gastrointestinal system.

The weight changes of male and female rats dosed with the same chemical/chemicals were compared using the Student's t-test and were considered significantly different at $p \leq .05$. A comparison of all treatment groups showed no statistically significant differences between weight changes of male and female rats.

During the dosing period, both male and female tetralin-exposed rats exhibited diarrhea. Additionally, the urine from both male and

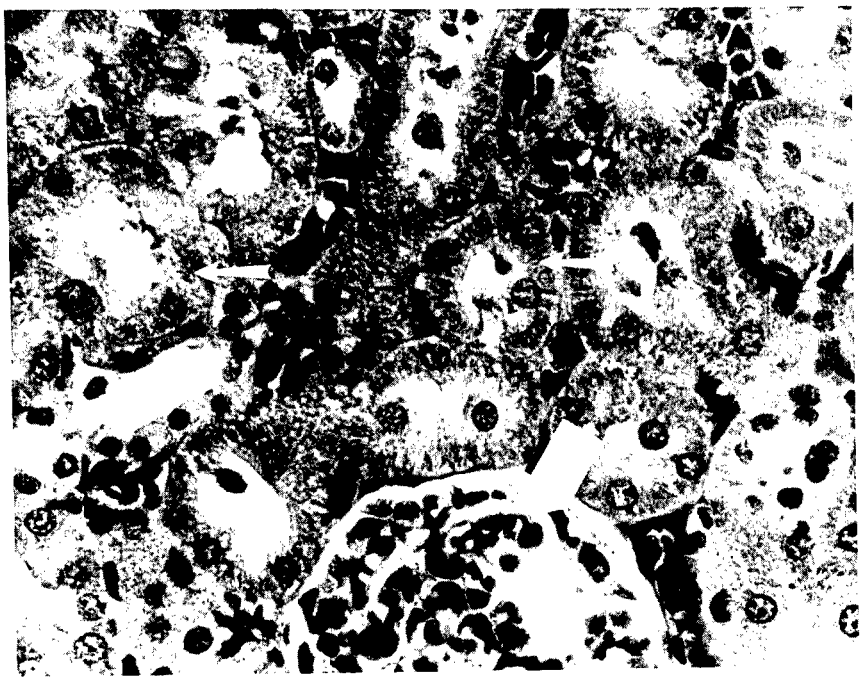
female rats displayed a dark-brown color. A test for occult blood and blood pigments was performed by means of a reagent strip and was negative. Therefore, the color of the urine was probably not the result of hematuria or hemoglobinuria. Additional tests to determine the cause of the urine color were not performed.

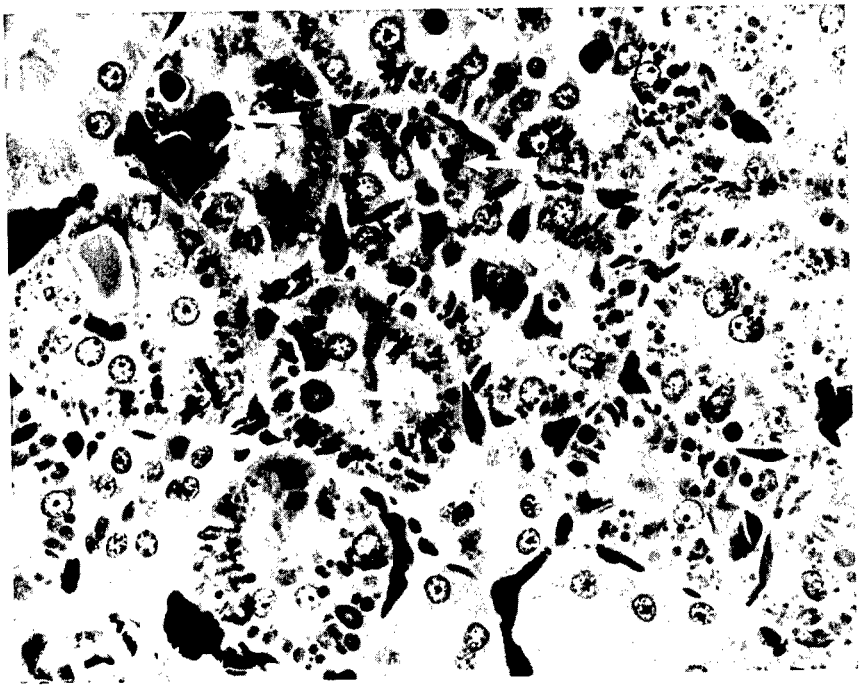
HISTOPATHOLOGY

Only male rats exposed to tetralin exhibited recognizable renal lesions. When compared with controls where minimal to mild hyaline droplets were observed, exposed males exhibited increased cytoplasmic hyaline droplets in proximal convoluted tubular epithelial cells. In non-pretreated exposed males the severity of hyaline droplet formation was regarded as moderate when hyaline droplet formation was graded on a scale where 0=no droplets, 1=minimal, 2=mild, 3=moderate, and 4=severe. Female control and exposed rats did not display hyaline droplet formation. The morphology of the droplets varied from elongated, crystalline forms to homogeneous spheroids of assorted dimensions. Additionally, foci of cellular degeneration were present within the proximal convoluted tubules of male rats and consisted of tubular segments where epithelial cells exhibited increased cytoplasmic basophilia and vesicular nuclei. Intratubular cellular casts, overt glomerular changes or significant inflammation was not seen. In rats pretreated with corn oil, sodium phenobarbital, and SKF 525-A, mild hyaline droplet accumulation was present. However, in 3-methylcholanthrene pretreated males, only one of three exposed rats exhibited renal hyaline droplet formation and these changes were regarded as mild. Hyaline changes and cellular degeneration found in kidney tissue of male rats exposed to tetralin were indicative of toxic injury. These renal lesions are shown in Figure 6 and described in relation to a control animal (Figure 5).

FIGURE 5. Renal Cortex of Control Male Rat. Large Arrow Identifies Glomerulus. Small Arrows Identify Cells of Proximal Convoluted Tubules Without Hyaline Droplet Formation (Magnification X 400 H & E).

FIGURE 6. Renal Cortex of Male Rat Exposed to Tetralin. Arrows Identify Marked Cytoplasmic Hyaline Droplets in Proximal Tubular Cells (Magnification X 400 H & E).





In rats pretreated with i.p. injections, varying degrees of liver capsulitis were observed and considered to be the result of the irritating properties of the chemicals (Bruner, personal communication). Histopathologic findings in liver tissue from non-pretreated rats were unremarkable.

As discussed in the introduction, several mechanisms may contribute to accentuated hyaline droplet formation and cellular degeneration within proximal convoluted tubules. Since findings suggest that alpha 2u globulin is the major constituent of hyaline droplets, the interaction of hydrocarbons or their metabolites with indigenous proteins like alpha 2u globulin or lysosomal enzymes might compromise protein catabolic pathways. Therefore, mechanisms involving hydrocarbons and their metabolites that cause this excessive protein accumulation within proximal tubular epithelial cells could be basic to the pathogenesis of hydrocarbon-induced nephropathy.

METABOLITE ANALYSIS

Possible alcohol and ketone metabolites were either purchased or synthesized and then analyzed by gas chromatography/mass spectrometry. To determine if detector response to these compounds was uniform, various known concentrations of each metabolite were analyzed by gas chromatography and the detector response, reported in peak area, determined. For each ketone and alcohol metabolite analyzed, the detector yielded a constant area count for a 1 ug sample of the metabolite. Hydroxyketones and diols could not be separated in pure form, and therefore, a precise detector response to these compounds could not be determined. However, after compensating for impurities, it can be estimated with some confidence that detector response to hydroxyketones and diols during urine and kidney extract analysis was also uniform.

Metabolites of Tetralin

Six tetralin metabolites were identified in the urine of male and female tetralin exposed rats. These were: 1-tetralol, 2-tetralol, 2-hydroxy-1-tetralone, 4-hydroxy-1-tetralone, 1,2-tetralindiol, and 1,4-tetralindiol. It is important to note that the urinary metabolites recovered following tetralin exposure were primarily disubstituted molecules whereas the metabolites identified from exposure to other cyclic hydrocarbons such as decalin and JP-10 have been monosubstituted molecules. Representative gas chromatographic tracings of urine samples from male rats treated with tetralin and from control rats are shown in Figure 7 and Figure 8, respectively. Mass spectra of synthesized tetralin metabolites are presented in Figure 9. Trace quantities of naphthol were also detected in male and female urine

FIGURE 7. GAS CHROMATOGRAPHIC TRACING OF URINE FROM MALE RATS DOSED WITH TETRALIN. TRACING IS REPRESENTATIVE OF MALE AND FEMALE RATS.

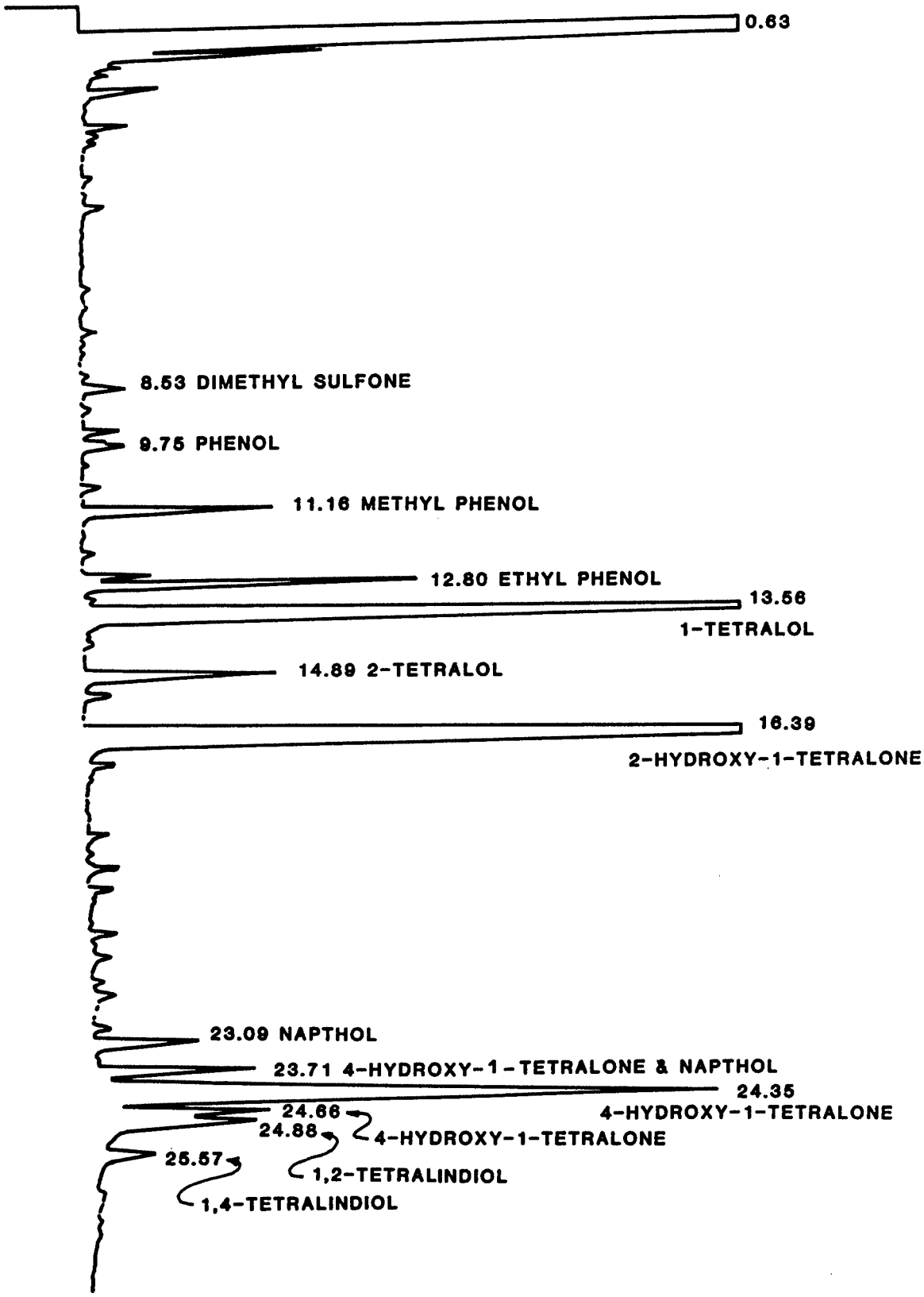


FIGURE 8. GAS CHROMATOGRAPHIC TRACING OF URINE FROM MALE RATS DOSED WITH WATER. TRACING IS REPRESENTATIVE OF BOTH MALE AND FEMALE RATS.

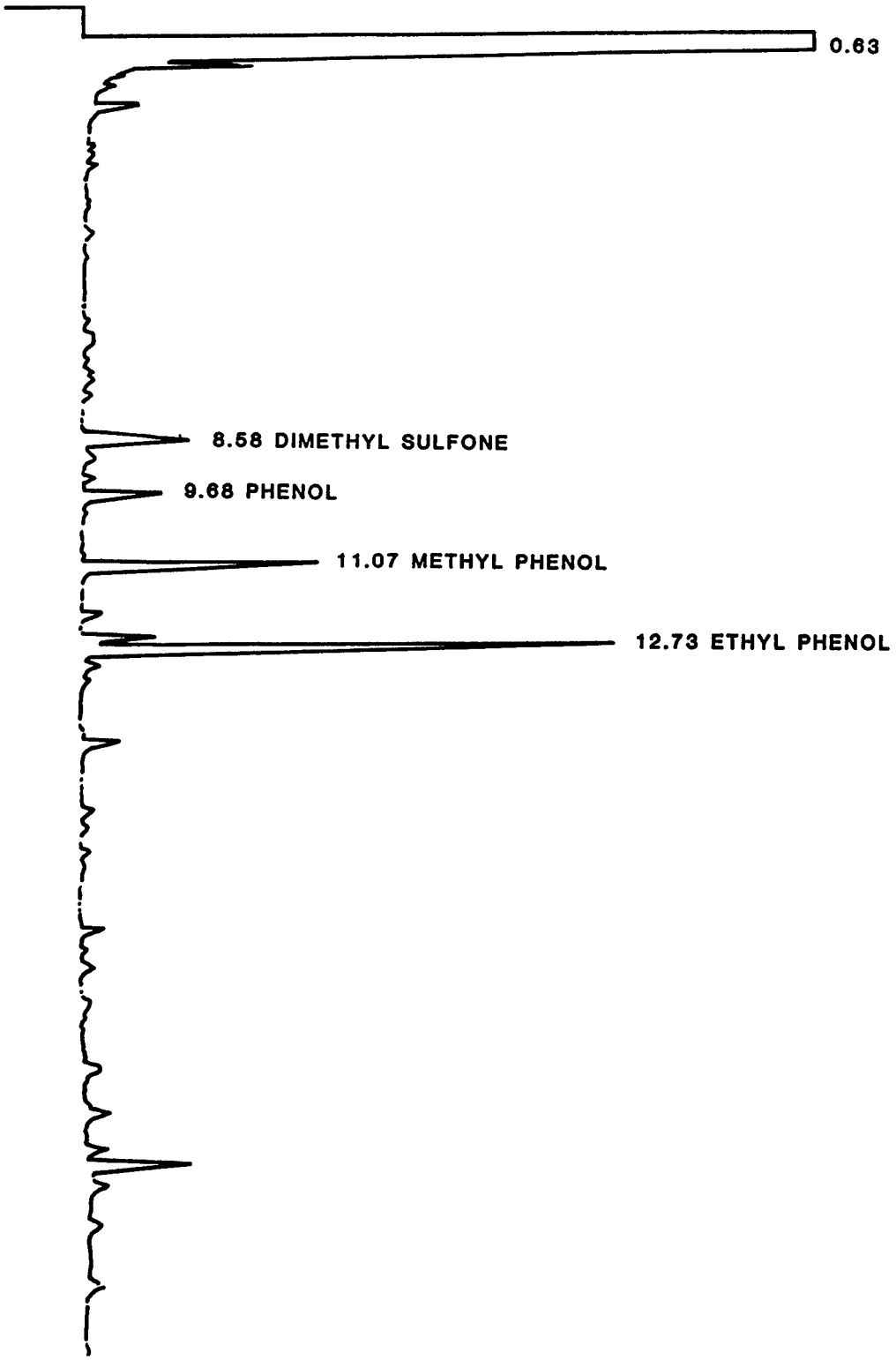
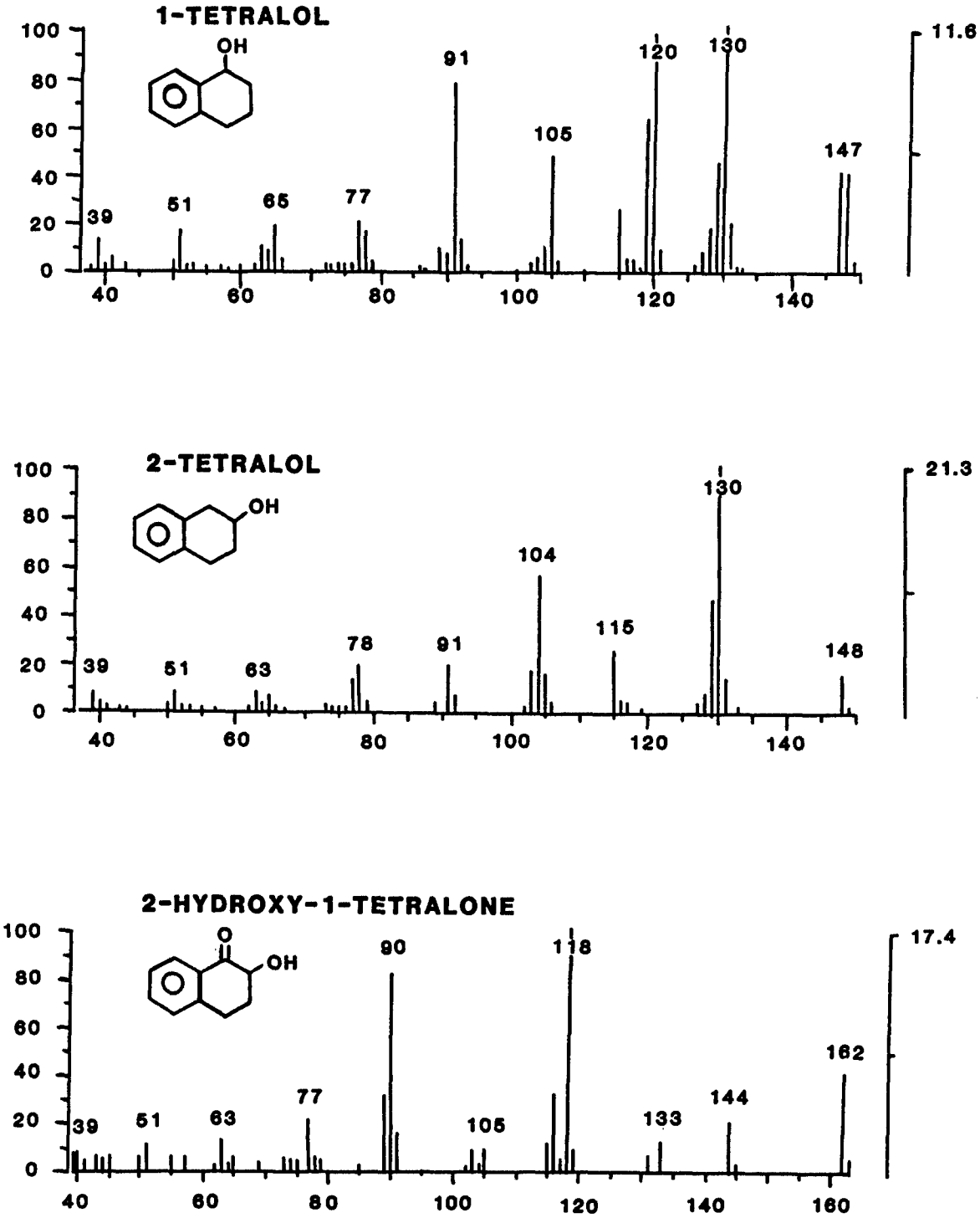
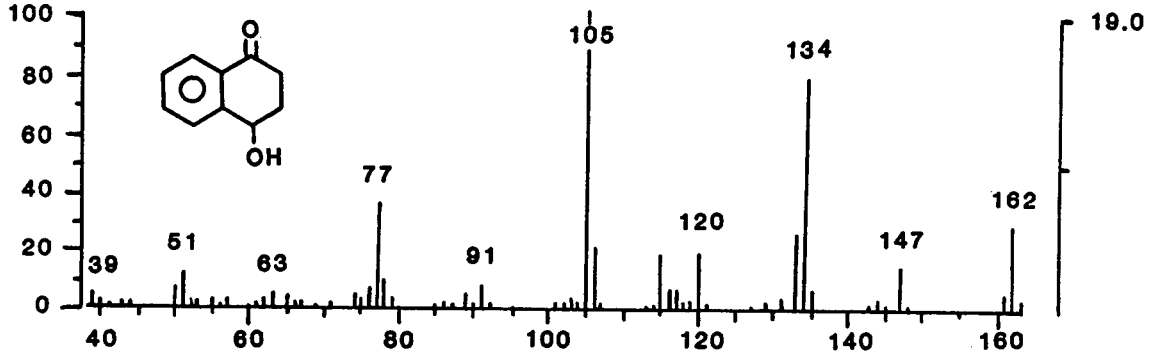


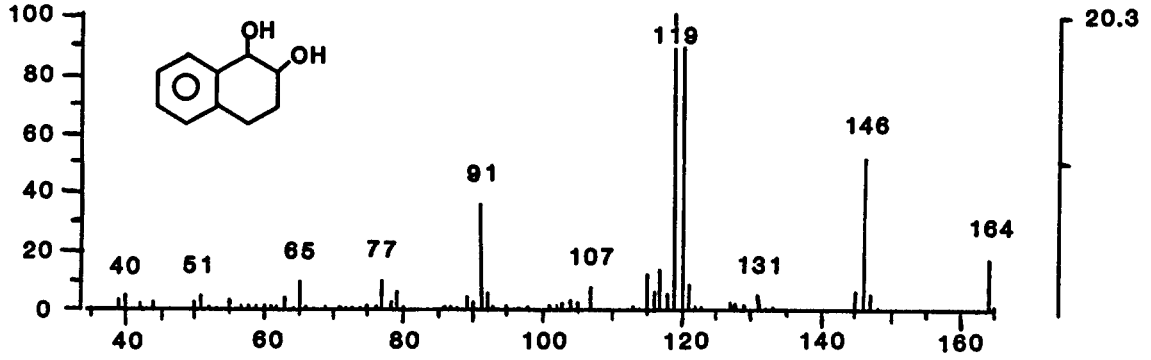
FIGURE 9. MASS SPECTRA OF SYNTHESIZED TETRALIN METABOLITES



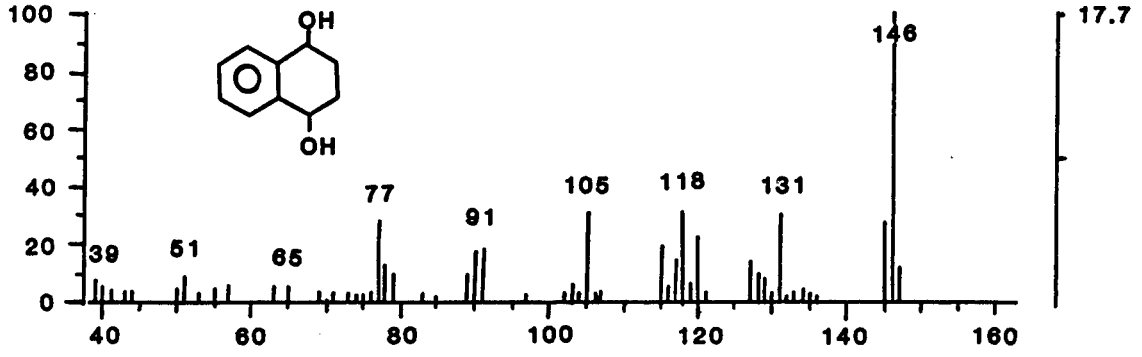
4-HYDROXY-1-TETRALONE



1,2 -TETRALINDIOL



1,4-TETRALINDIOL



samples but were considered to be the metabolite of the minor naphthalene impurity present in the tetralin used for dosing.

Relative amount percentages of identified metabolites for male and female rats dosed with tetralin are shown in Table 8. Because of inconsistent resolution during gas chromatographic detection, relative amount comparisons could not be accurately determined for 1,2-tetralindiol in male and female rats and for 1,4-tetralindiol and final 14th day urine amounts of 4-hydroxy-1-tetralone in female rats. Also, three peaks were identified by GC/MS that contained 4-hydroxy-1-tetralone. The largest peak was pure 4-hydroxy-1-tetralone with the other two peaks containing small amounts of 4-hydroxy-1-tetralone, a naphthol and 1,4-tetralindiol. The major 4-hydroxy-1-tetralone peak was used for relative amount comparisons.

The major tetralin metabolites in urine for both male and female rats were 1-tetralol, 2-hydroxy-1-tetralone, and 4-hydroxy-1-tetralone. The relative amount percentages of metabolite recovered for male and female rats were statistically compared for each collection period. Values were considered significantly different at $p \leq .05$ using the Student's t-test. In each case where there was a significant difference, a greater percentage of the metabolite was present in the female rat urine. Relative amounts of 2-tetralol were significantly greater in female rats for 24 hr, 48 hr and final 14th day urine collections. The relative amount of 2-hydroxy-1-tetralone was significantly greater in female rats for 24 hr and final 14th day collections while 1-tetralone was greater for only the final collection period.

TABLE 8. RELATIVE AMOUNT COMPARISONS OF TETRALIN METABOLITES IDENTIFIED IN URINE OF MALE AND FEMALE RATS EXPOSED TO TETRALIN

METABOLITES	RELATIVE AMOUNT OF METABOLITE RECOVERED PER COLLECTION PERIOD (Percentage) [a,b]					
	24 HR		48 HR		FINAL[c]	
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
1-Tetralol	24 ± 3 (6)	27 ± 3 (6)	27 ± 6 (6)	34 ± 3 (6)	26 ± 2 (6)[e]	37 ± 7 (6)[e]
2-Tetralol	4 ± 1 (6)[f]	8 ± 1 (6)[f]	4 ± .5 (6)[g]	8 ± 1 (6)[g]	4 ± .3 (6)[h]	14 ± 4 (6)[h]
2-Hydroxy-1-tetralone	29 ± 4 (6)[i]	35 ± 2 (6)[i]	33 ± 5 (6)	31 ± 2 (6)	30 ± 2 (6)[j]	42 ± 9 (6)[j]
4-Hydroxy-1-tetralone	33 ± 4 (6)	30 ± 5 (6)	27 ± 2 (6)	28 ± 4 (6)	32 ± 4 (6)	.[d]
1,4-tetralindiol	1 ± .5 (6)	.[d]	2 ± .3 (6)	.[d]	2 ± 1 (6)	.[d]
1,2-tetralindiol	.[d]	.[d]	.[d]	.[d]	.[d]	.[d]

a = Mean ± S.D. (N)

b = Percentages are representative of the relative amount of each metabolite as compared to total area of metabolites listed

c = Overnight urine collections following last dosing

d = Detectable, but unable to resolve and quantitate

e, f, g, h, i, j = The relative amount percentages of metabolite recovered for male and female rats were compared for each collection period. Values with identical superscripts were significantly different from each other a $p \leq .05$ using the Student's t-test.

Tetralin metabolites were not detected during GC/MS analyses of kidney extracts from tetralin-exposed male and female rats. The presence of renal lesions in the kidneys of male rats exposed to tetralin with the absence of tetralin metabolites in the kidney extracts is not in agreement with findings from previous cyclic hydrocarbon studies. In studies where male and female rats were dosed with decalin or JP-10, male rats that exhibited renal damage also had ketone metabolites present in their kidney extracts. However, female rats did not exhibit renal damage and metabolites were not detected in their kidney extracts. In tetralin-dosed female rats, the lack of both renal damage and kidney extract metabolites is in agreement with previous hydrocarbon studies. The absence or presence of tetralin metabolites in urine and kidney extracts for all exposure groups and the corresponding renal pathology is summarized in Table 9.

Metabolites of Rats Given Enzyme Modifiers

Xenobiotics are metabolized by enzyme systems present predominantly in the liver; however, kidney, lung, brain, intestine, and skin also contain these enzyme systems (Estabrook, 1982). These systems are collectively known as the mixed function oxidase system (MFO) and serve to increase the water solubility of the xenobiotic and speed its elimination from the body. The terminal oxidase of the MFO system is a group of hemeproteins called P-450. During metabolism, many xenobiotics can inhibit or induce the capacity of the MFO system to metabolize foreign compounds. Metabolic conversion of a xenobiotic can result in the formation of products that are either less toxic or more toxic than the parent compound. Therefore, when metabolism decreases toxicity, enzyme induction can be protective and enzyme

inhibition detrimental or conversely, when metabolism increases toxicity, enzyme inhibition can be protective and enzyme induction detrimental.

To determine what effects inhibition or induction of the MFO system might have on tetralin metabolism and toxicity, enzyme modifiers were given to rats prior to tetralin exposure. The three enzyme modifiers administered were phenobarbital, 3-methylcholanthrene, and SKF 525-A. Rats pretreated with corn oil served as controls.

Phenobarbital, which is actively metabolized by the liver MFO, increases the amount of cytochrome P-450 and other associated MFO enzymes in the liver cell (Hodgson, 1982). However, phenobarbital does not induce renal MFO activity in the rat or mouse. Pretreatment of rats with 3-methylcholanthrene causes an increase in hepatic and renal cytochrome P-448 levels (Casarett and Doull, 1980). Finally, SKF 525-A interferes with the binding of substrates to cytochrome P-450 and reduces the rate of drug metabolism (Goodman and Gilman, 1980). The absence or presence of tetralin metabolites in urine and kidney extracts for all exposure groups and the corresponding renal pathology is summarized in Table 9.

Relative amounts of tetralin metabolites for positive control male rats pretreated with corn oil before tetralin dosing and male rats pretreated with sodium phenobarbital and SKF 525-A compared closely with male rats exposed only to tetralin. However, the relative amounts of metabolites detected from male rats pretreated with 3-methylcholanthrene were different than those from other pretreated male rats and controls. All urine samples from 3-methylcholanthrene pretreated male rats had lower amounts of 2-hydroxy-1-tetralone and slightly increased

levels of 4-hydroxy-1-tetralone in 48 hour and final two-week urine samples when compared to positive controls and other pretreated male rats. There was also an increase in the levels of 1,2-tetralindiol in all urine samples from 3-methylcholanthrene pretreated male rats.

When compared to female tetralin-exposed rats, female positive control rats had more 4-hydroxy-1-tetralone in their urine samples. Female rats pretreated with SKF 525-A had similar relative amounts of metabolites as did the female positive control rats except for a slight increase in 2-hydroxy-1-tetralone. Compared to female positive control rats, female rats pretreated with sodium phenobarbital showed a decrease in the relative amounts of 2-tetralol and 4-hydroxy-1-tetralone in 24 hr and 48 hr urine samples. 3-Methylcholanthrene pretreated female rats had decreased levels of 2-tetralol in all urine samples and decreased 2-hydroxy-1-tetralone in their 48 hr urine samples but in contrast had increased levels of 4-hydroxy-1-tetralone in their 48 hr and final urine samples.

Tetralin metabolites were not detected during GC/MS analysis of the kidney extracts taken from female positive control rats or female rats pretreated with sodium phenobarbital, 3-methylcholanthrene or SKF 525-A. However, tetralin, 1-tetralol, 2-tetralol, and 1-tetralone were detected in the extracts from positive control male rats and male rats pretreated with sodium phenobarbital and SKF 525-A. The appearance of renal lesions and a ketone metabolite in kidney extracts of pretreated male rats is noteworthy when non-pretreated male rats that also exhibited renal lesions had no ketone metabolite present in their kidney extracts. A representative gas chromatographic tracing of kidney extracts from these rats is shown in Figure 10. The mass

FIGURE 10. REPRESENTATIVE GAS CHROMATOGRAPHIC TRACING OF URINE FROM MALE RATS PRETREATED WITH SODIUM PHENOBARBITAL AND THEN DOSED WITH TETRALIN

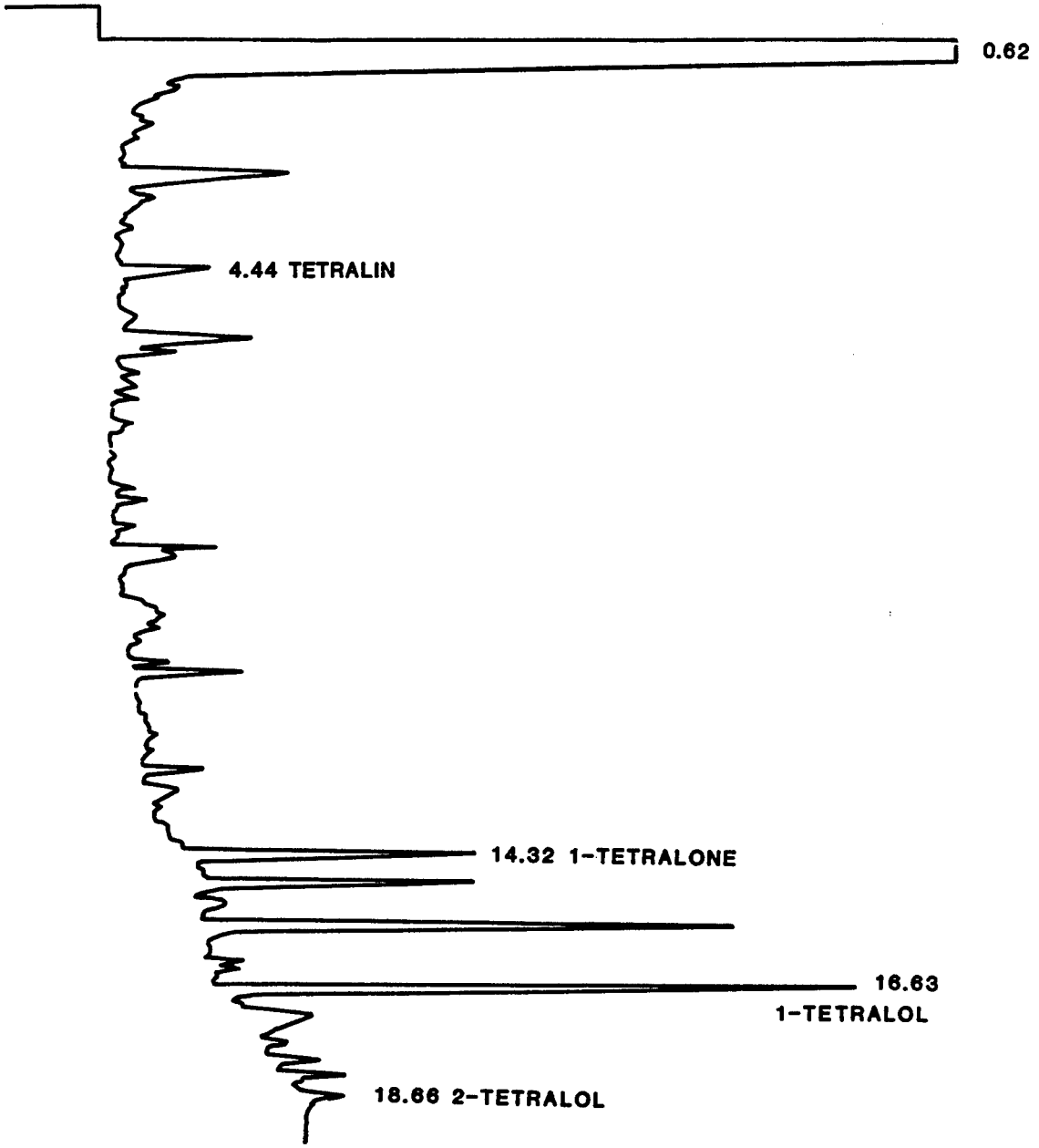
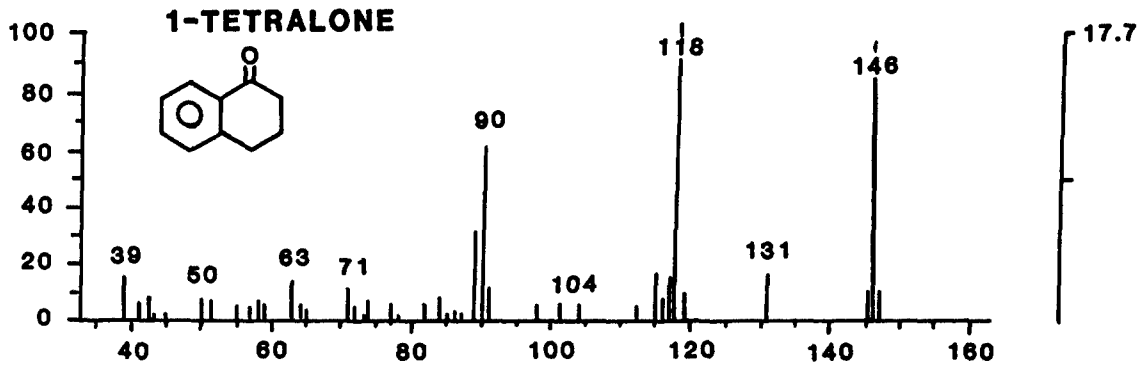


FIGURE 11. MASS SPECTRUM OF SYNTHESIZED 1-TETRALONE



spectrum fragmentation pattern of synthesized 1-tetralone is presented in Figure 11. One male rat pretreated with sodium phenobarbital and one male rat pretreated with SKF 525-A showed no tetralin or 2-tetralol in their kidney extracts, but did have 1-tetralol and 1-tetralone. One male rat pretreated with 3-methylcholanthrene had only 1-tetralol and 1-tetralone detected in its kidney extract. This male rat was also the only 3-methylcholanthrene pretreated rat to exhibit renal lesions. Tetralin metabolites were not detected in the two 3-methylcholanthrene pretreated rats which did not demonstrate renal lesions. Because these tetralin metabolites were present in kidney extracts in only small amounts, consistent gas chromatographic detection of them was difficult. Therefore, for the animals in which metabolites were detected, only relative amount comparisons of the major metabolites, 1-tetralol and 1-tetralone, were performed. For positive control male rats and male rats pretreated with sodium phenobarbital or SKF 525-A, the relative amount percentages of 1-tetralol and 1-tetralone were 49.8 $\pm$ 7.78 (9) and 50.2 $\pm$ 7.78 (9), respectively. Although tetralin metabolites were present in the kidney extract of one male rat pretreated with 3-methylcholanthrene, it is interesting to note the relative amounts of 1-tetralol and 1-tetralone were 82% and 18%, respectively. Differences between the type of metabolite detected in kidney extracts of pretreated and non-pretreated male rats, and the absence of renal lesions and metabolites in two 3-methylcholanthrene pretreated male rats, suggest induction or that inhibition of the MFO enzyme system affects the metabolism of tetralin and possibly alters its toxicity. Therefore, the investigation of cyclic hydrocarbon metabolism following enzyme modification should be continued.

TABLE 9. TETRALIN METABOLITES DETECTED IN URINE AND KIDNEY EXTRACTS AND CORRESPONDING RENAL PATHOLOGY

GROUP & TREATMENT	EXPOSURE ROUTE AND DOSE	URINARY METABOLITES		KIDNEY METABOLITES		RENAL LESIONS	
		MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
1. Negative Control Water	0.5 mL/kg i.g.	NO	NO	NO	NO	YES (Min/Mild)	NO
2. Tetralin	0.5 mL/kg i.g.	YES[a]	YES[a]	NO	NO	YES (Moderate)	NO
Enzyme Modifiers							
3. Positive Control Corn Oil Tetralin	2.0 mL/kg i.p. 0.5 mL/kg i.g.	YES[a]	YES[a]	YES[b]	NO	YES (Mild)	NO
4. Sodium Phenobarbital Tetralin	102 mg/kg i.p. 0.5 mL/kg i.p.	YES[a]	YES[a]	YES[c]	NO	YES (Mild)	NO
5. SKF 525-A Tetralin	25 mg/kg i.p. 0.5 mL/kg i.g.	YES[a]	YES[a]	YES[d]	NO	YES (Mild)	NO
6. 3-MC Tetralin	27 mg/kg i.p. 0.5 mL/kg i.g.	YES[a]	YES[a]	YES[e]	NO	YES (Mild)[e]	NO

a = 1-tetralol, 2-tetralol, 2-hydroxy-1-tetralone, 4-hydroxy-1-tetralone,1,2-tetralindiol and 1,4-tetralindiol
b = tetralin, 1-tetralol, 2-tetralol and 1-tetralone
c = 1 rat had 1-tetralol and 1-tetralone, 2 rats had tetralin, 1-tetralol, 2-tetralol and 1-tetralone
d = 1 rat had 1-tetralol and 1-tetralone, 2 rats had tetralin, 1-tetralol, 2-tetralol and 1-tetralone
e = 1 rat had 1-tetralol and 1-tetralone and exhibited renal lesions the other two rats had no metabolites and no renal lesions

IV. CONCLUSION

This research was initiated to provide toxicity information regarding the nephrotoxic hazards of cyclic hydrocarbons. Because of its unique structure, tetralin was chosen as the test compound. When compared with control rats, only male rats exposed to tetralin exhibited recognizable renal lesions. This study contributed additional insight into the effects of compound structure and activity relationships on nephrotoxicity. Specific findings suggest that the structural and/or electronic character of tetralin did not prevent the occurrence of nephrotoxicity in male rats. Male and female rats dosed with tetralin produced the same urinary metabolites, which were primarily disubstituted molecules. Finally, while tetralin metabolites were not detected in the kidney extracts of either male or female tetralin-dosed rats, pretreatment with enzyme modifiers produced detectable ketone and alcohol metabolites in the kidney extracts of male rats and, in the case of two 3-methylcholanthrene pretreated rats, appeared to have inhibited renal lesions. No correlation can be made between the type of metabolite recovered and the severity of hyaline droplet accumulation.

The observation of nephrotoxicity in male rats exposed to tetralin raises important questions regarding the biohazardous potential of many cyclic hydrocarbon fuels. Fundamental to hazard assessment is the observation that tetralin, like many hydrocarbons, failed to induce renal lesions in female rats. This sex-dependent toxic effect supports evidence that the male rat is physiologically predisposed to the renal

effects of certain hydrocarbons and may not be an appropriate model for human risk assessment. If metabolic pathways reponsible for hydrocarbon nephrotoxicity in male rats are explained, and found to be absent in humans, many important fuels may be exonerated as potential health hazards. In order to characterize more fully the nature of hydrocarbon-induced nephrotoxicity, further research should be conducted.

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